The fibre diffraction pattern of microtubules.

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Microtubules are long flexible macromolecular assemblies which constitute, among else, part of the cyto-skeleton and the mitotic spindle. A microtubule is a hollow cylindrical structure of 30 nm diameter and a wall thickness of 8 nm. The microtubule wall consists of tubulin heterodimers, which are assembled in a head to tail fashion, into the so called protofilaments. Thirteen protofilaments connect laterally to form the hollow microtubule. The protofilaments are slightly staggered with respect to each other. See figure 1.

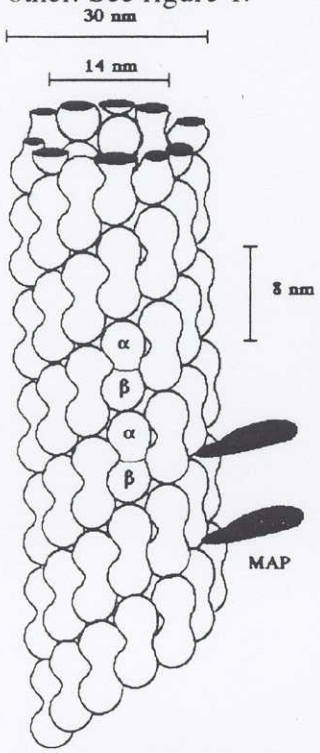

Figure 1 Schematic drawing of an assembled microtubule. The α–β heterodimer is indicated as well as the two microtubule associated proteins which stick out of the microtubule wall and are thought to be essential for the functioning of the microtubules in the cell.

Several attempts to obtain fibre diffraction patterns from microtubules have been reported. Unfortunately the results are of low quality or the degree of hydration is disputable. The most succesful method for orienting the samples sofar has been to centrifuge them at high speeds over extended periods (24 hours). A possible alternative method might be to shear the sample material. Oriented samples have indeed been produced in this way but the uniform shear field essential for the orientation of the samples could only be obtained with a Couette type of shear cell which renders this method unusable for the preparation of X-ray fibre diffraction samples.

We have developed an alternative orientation method. By assembling the samples in a strong magnetic field (>7 Tesla) it was shown to be possible to obtain a high degree of orientation. It is thought that this is possible due to the fact that the small diamagnetic moment of the α-helical components of the tubulin heterodimer has a net component along the dimer axis. When the microtubules assemble these diamagnetic moments will be vectorially added so that a larger total diamagnetic moment is created. The success of this method is heavily dependent on the buffer conditions, the protein concentration and the strength of the magnetic field. It is only shown to be possible to obtain good orientation in solutions of up to 9 mg/ml in a heavily scattering, glycerol assembly buffer. Experimental difficulties are the high radiation sensitivity of the samples and the relatively low concentration, which means that the data obtained on several samples, over several days, have to be averaged. This is a severe test for the stability of the detector systems and it has been shown that the data collected suffers more from drift in the detector performance than from low statistical accuracy. The fibre diffraction pattern obtained in this way shows that there still is a considerable angular spread in the microtubule axes. See figure 2. A fortunate side effect of the relatively large arcing of the diffraction features is that the errors introduced by detector drifts are, to a large extent, averaged out.

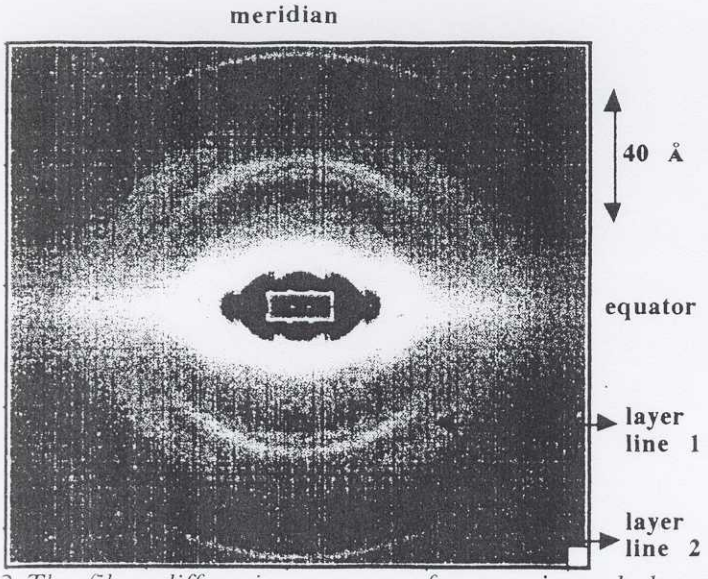

Figure 2 The fibre diffraction pattern of pure microtubules assembled in a 9 Tesla field. The data is the average of data collected on 8 individual samples.Total data collection time 3.5 hours, collected over a period of three days. Average protein concentration 7.5 mg/ml.

In order to obtain reliable diffraction information it was necessary to correct the diffraction pattern for the effects of the angular spread. To achieve this the diffraction pattern was first remapped onto the diffraction sphere using the program FTOREC. After this transformation was applied the program LSQUINT was used to calculate what the effect was from the angular spread on the diffraction features. This enabled us to determine what the diffraction intensities on the equatorial axis and the first layer line were. An important result from this was that some intensitiy maxima, observed in the solution scattering pattern, could be shown to be due to higher order repeats from Bessel functions contributing to the equator instead of being due to the first layer line bessel function contributions. These higher orders have not been seen before (by other authors) and are not clearly visible in the uncorrected pattern. See figure 3.

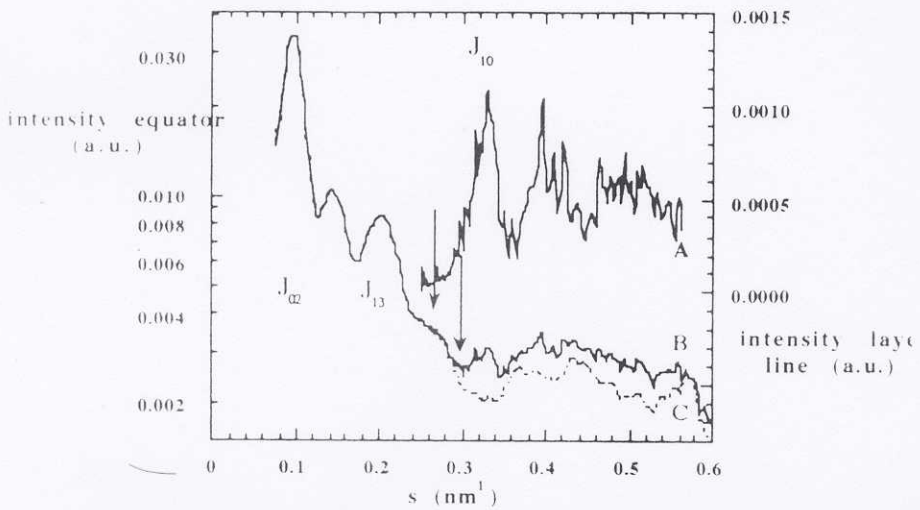

Figure 3 Diffraction intensities on the equator (C), the projection of the first layer line onto the equator (A) and the simulated solution scattering pattern (B). The position of the J_3 contributions, which are clearly dominated by the higher order maxima of the equator, are indicated with the arrows.

However, due to the angular spread, the program LSQUINT interprets data near the meridional axis, on the first layer line as true meridional reflections. Since the microtubules have a helical symmetry this is not realistic. Therefore we investigated

the possibility of treating the first layer line with an intelligent procedure of Fourier-Bessel smoothing. By forcing the fitting procedures to obey the rules of helical diffraction it was possible to resolve individual J_3 Bessel function contributions arising from the inside as well as outside wall of the microtubule (see Fig. 4). The fact that the microtubule wall dimensions calculated from these J_3 Bessel functions are in good agreement with the dimensions determined by alternative methods reinforces the belief that the procedure is valid.

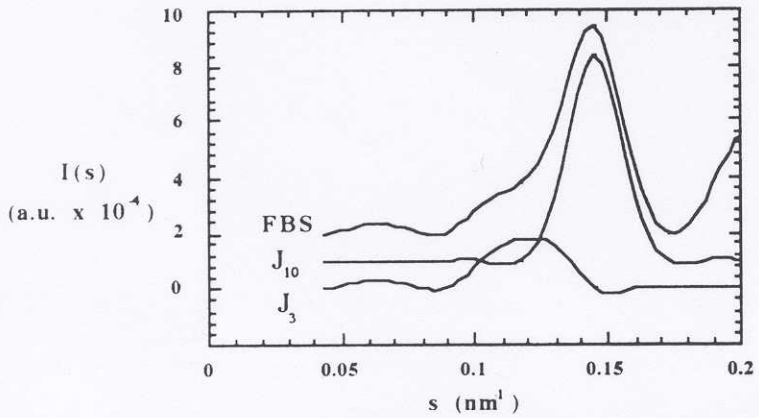

Figure 4 Fourier-Bessel smoothed near meridional first layer line diffraction intensity. In the original data set the two J_3 functions arising from respectively the outside and the inside microtubule wall could not be resolved in the unprocessed data.

We have shown that by intelligent use of CCP13 software it is possible to extend the possibilities of data analysis, even with rather disoriented specimens such as microtubules, in an apparently sensible way.